

## APPENDIX D

### All Pending Claims 1-20

1. An oligonucleotide which hybridizes to a non-transcribed spacer sequence between rRNA genes of an organism of the genus *Perkinsus* being assayed, wherein said organism of genus *Perkinsus* contains a nucleotide base sequence selected from the group consisting of the sequences shown in Fig 2, 3, 4 and 17.
2. A method of making an oligonucleotide for use in assaying a target organism of the genus *Perkinsus* comprising the steps of:
  - (i) extracting DNA from said target organism
  - (ii) isolating from said DNA a non-transcribed spacer sequence flanked by rRNA genes;
  - (iii) sequencing said non-transcribed spacer sequence; and
  - (iv) synthesizing an oligonucleotide having a nucleic acid sequence as shown in Fig 17.
3. A kit for determining the identity of species of a microorganism of the genus *Perkinsus*, comprising a container having outwardly directed PCR primer pairs to a nontranscribed spacer sequence flanked by rRNA genes, said primer pairs, having a nucleic acid sequence selected from the group consisting of sequences shown in Figs. 2, 3, 4 and Fig. 17.
4. The oligonucleotide of claim 1 wherein said organism is *Perkinsus atlanticus*.
5. The oligonucleotide of claim 4 wherein said nucleotide base of said organism sequence is shown in Fig. 17.
6. The oligonucleotide of claim 1 wherein said organism is *Perkinsus andrewsi*.

7. The oligonucleotide of claim 6, wherein said nucleotide base sequence of said organism is shown in Fig. 3.

8. The oligonucleotide of claim 1, wherein said organism is *Perkinsus mackini*.

9. The oligonucleotide of claim 1 wherein said oligonucleotide is one of a pair of PCR primers, or complement thereof.

10. The oligonucleotide of claim 9, wherein said oligonucleotide is between about 10 to 35 nucleotides in length.

11. The oligonucleotide of claim 9, wherein said oligonucleotide is between about 15 to 24 nucleotides in length.

12. The oligonucleotide of claim 9 wherein said PCR primers or complement thereof are selected from the group consisting of:

CAC TTG TAT TGT GAA GCA CCC

TTG GTG ACA TCT CCA AAT GAC

ATG CTA TGG TTG GTT GCG GAC C

GTA GCA AGC CGT AGA ACA GC

AAG TCG AAT TGG AGG CGT GGT GAC

ATT GTG TAA CCA CCC CAG GC

TAG TAC CCG CTC ATT GTG G

TGC AAT GCT TGC GAG CT

AGT TGG ATT TCT GCC TTG GGC G

ACC AGG TCC AGA CAT AGG AAG G

13. The oligonucleotide of claim 1, wherein said oligonucleotide is detectably labeled.

14. The oligonucleotide of claim 1, wherein said nucleotide base sequence has type I and type II NTS sequences as in Fig. 10.

15. The oligonucleotide of claim 1, wherein said nucleic acid sequence is exactly complementary to said nontranscribed spacer sequence.

16. The method of claim 2, wherein said nontranscribed spacer sequence is isolated by amplifying said nontranscribed spacer sequence using primers, or complement thereof that preferentially hybridize to said flanking rRNA genes.

17. The method of claim 2, wherein said nontranscribed spacer is isolated by the steps of digesting said DNA with restriction enzyme, creating a library, and identifying said nontranscribed spacer sequences within said library using a probe specific for one of said rRNA genes.

18. The method of claim 2, wherein said oligonucleotide is one of a pair of PCR primers or complement thereof.

19. The kit of claim 3, wherein said microorganism is the genus *Perkinsus*.

20. The kit of claim 3 wherein said PCR primers pairs or complement thereof are selected from the group consisting of sequences as shown in Figs. 20 and 21.